Effects of Snowdrop Lectin (*Galanthus nivalis* Agglutinin) Expressed in Transgenic Sugarcane on Fitness of *Cotesia flavipes* (Hymenoptera: Braconidae), a Parasitoid of the Nontarget Pest *Diatraea saccharalis* (Lepidoptera: Crambidae)

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ABSTRACT *Cotesia flavipes* (Cameron) is a parasitoid responsible for maintaining populations of sugarcane borer, *Diatraea saccharalis* (F.), below economic levels in south Texas sugarcane fields. Transgenic sugarcane expressing the snowdrop lectin (*Galanthus nivalis* agglutinin, GNA) was developed against the Mexican rice borer, *Eoreuma loftini* (Dyar), the primary pest of south Texas sugarcane. The potential impact of GNA-expressing sugarcane on various biological and fitness parameters of *Cotesia flavipes* (Cameron) was studied in the laboratory to gain insight on likely effects of the transgenic sugarcane on biological control of sugarcane borer by *C. flavipes.* Females of *C. flavipes* were offered sugarcane borer larvae fed one of two diet treatments for oviposition for two successive generations: (1) artificial diet containing transgenic sugarcane tissue or (2) artificial diet containing nontransgenic sugarcane tissue. Small to marginal negative effects of artificial diet containing transgenic sugarcane tissue were evident in the rate of host suitability, number of cocoons and adult parasitoids emerging per host, percentage cocoons yielding parasitoids, and sex ratio and adult lifespan of parasitoids. These effects were variable between the two parasitoid generations examined. In contrast, differences were not detected between diet treatments in rates of host acceptance, egg load of females, and egg to adult developmental periods. The negative effects of transgenic sugarcane on *C. flavipes* detected in this study are important because GNA levels in the diet (≈0.49% of total protein content) containing transgenic sugarcane tissue were ≈50% of the level expressed in transgenic sugarcane plants. Results are discussed in relation to potential impacts of the transgenic cultivar on biological control of sugarcane borer by *C. flavipes.*

KEY WORDS *Cotesia flavipes*, *Diatraea saccharalis*, *Galanthus nivalis* agglutinin, biological control, nontarget effects, development

IN RECENT YEARS, genetically engineered crop cultivars resistant to insect pests have attracted much attention. Transgenic sugarcane expressing snowdrop lectin (*Galanthus nivalis* agglutinin, GNA) was developed at the Texas Agricultural Experiment Station, Weslaco, against the Mexican rice borer, *Eoreuma loftini* (Dyar) (Lepidoptera: Crambidae), which is currently the primary pest of sugarcane in south Texas (Irvine and Mirkov 1997, Legaspi et al. 1997). Lectins such as GNA have been suggested to have toxic effects toward insect pests, especially lepidopteran larvae, although apparently they have low to nil mammalian toxicity (Czapla and Lang 1990, Pusztai et al. 1996, Fitches et al. 1997, Ewen and Pusztai 1999). The Mexican rice borer became the primary pest of sugarcane in south Texas following successful biological control of sugarcane borer, *Diatraea saccharalis* (F.), by *Cotesia flavipes* (Cameron) in the early 1980s (Legaspi et al. 1997, Meagher et al. 1998). *Cotesia flavipes* remains the most important natural mortality factor of *D. saccharalis* in south Texas, consistently maintaining populations of this pest below economic levels (Fuchs et al. 1979, Legaspi et al. 1997, Meagher et al. 1998).

Insect-resistant crop cultivars, including transgenic crops, must be compatible with other pest management tactics to be effective and sustainable. In particular, transgenic cultivars should not interfere with biological control of secondary pests that are tolerant of the transgenic cultivar. Thus, far, laboratory studies have shown significant negative effects of transgenic sugarcane tissue, delivered via artificial diet, on life history parameters of Mexican rice borer, the target pest of transgenic sugarcane in south Texas, while nil to positive effects were evident in the case of sugarcane borer (unpublished data). The effects on Mexican rice borer were as anticipated, and it is likely that GNA-expressing sugarcane can contribute to reducing damage caused by this pest in the field. However, the nil to positive effects of the transgenic sugarcane on

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sugarcane borer raised concerns over the possibility that if *C. flavipes* is more susceptible to GNA than sugarcane borer, then biological control of this pest may be compromised with deployment of the GNA-expressing cultivar.

The effect of transgenic plants on nontarget organisms such as parasitoids has been addressed in a number of studies (Bell et al. 1999, Schuler et al. 1999, Hilbeck et al. 2000). Few and inconsistent negative effects of GNA, delivered via artificial diet or transgenic plants, were evident on fitness parameters of *Eulophus pennicornis* (Nees), a gregarious ectoparasitoid of larvae of the tomato moth *Lacanobia oleracea* (L.) (Bell et al. 1999). However, the biology of *E. pennicornis* may preclude the occurrence of significant negative effects; *E. pennicornis* is an idiobiont (hosts are either in nongrowing stages or their growth is inhibited by parasitization), and feeding and development in parasitized hosts are severely restricted due to envenomization by ovipositing parasitoids (Marris and Edwards 1995, Weaver et al. 1997). Birch et al. (1999) reported that GNA transgenic potato resistant against *M. persicae* adversely affected fecundity, egg viability, and longevity of twospotted lady beetle, *Adalia bipunctata* (L.). In contrast, no acute toxicity of GNA was noted when feeding twospotted lady beetle larvae on aphids, *Myzus persicae* (Sulzer), that had previously ingested GNA (Down et al. 2000). Finally, direct and indirect sublethal effects of GNA on the development of the potato-aphid parasitoid *Aphelinus abdominalis* have been recorded (Couty et al. 2001). Hence, host-mediated effects of GNA-expressing plants on natural enemies are likely to occur and merit examination, particularly in cases where herbivores feeding on these plants are tolerant of GNA and kept below economic levels by natural enemies with unknown tolerance toward GNA.

The overall goal of this study was to address potential host-mediated effects of GNA-expressing transgenic sugarcane on *C. fla*vipes under laboratory conditions before wide-scale deployment of this genetically engineered cultivar. Such an evaluation is critical due to the established regulatory effect of *C. flavipes* on sugarcane borer populations in south Texas (Wiedenmann and Smith 1993, Meagher et al. 1998). To date, no studies have addressed potential host-mediated effects of transgenic sugarcane on parasitoids of sugarcane pests. The specific objectives of this study were to evaluate the potential effects of transgenic sugarcane expressing GNA, delivered via artificial diet, on various biological and fitness parameters of *C. flavipes.*

Materials and Methods

Artificial Diets. Two types of diets were used in the study. Both were based on an artificial diet used regularly for rearing stalkborers in our laboratory (Martinez et al. 1988). This artificial diet was supplemented with leaf sheath tissue from either (1) nontransgenic (cultivar 'CP 65–357') or (2) transgenic (cultivar 'CP 65–357', transgenic line 83) sugarcane expressing ≈0.89% of GNA in the total extractable protein (unpublished data). The GNA-producing gene was introduced from snowdrop lily (*Galanthus nivalis*) into the sugarcane (cultivar CP65–357) using maize ubiquitin as promoter and the paint-sprayer delivery technique (Irvine and Mirkov 1997). The concentration of GNA in transgenic plant and diet were determined using western blot and immunostaining procedures (unpublished data). Leaf sheath tissue was added at a concentration of 10 g of tissue per 150 g of total diet weight, resulting in mean total extractable protein of 0.32 μg/μl and 0.35 μg/μl in the nontransgenic and transgenic diet treatments, respectively (unpublished data). Both diets were offered to sugarcane borer larvae in small plastic cups at a rate of ≈5 g diet per cup.

Insects. Sugarcane borer larvae used in the experiments originated from a laboratory colony of over 5,000 individuals per generation maintained at the Texas Agricultural Experiment Station, Weslaco. Insects collected from sugarcane stalks in the field were maintained for <1 yr on meridic diet as described previously (unpublished data). Field-collected individuals were incorporated regularly in the colony to reduce inbreeding. Newly laid eggs were collected from the colony and transferred to glass jars for incubation. The jars contained tap water saturated with NaCl as a source of humidity. Eggs inside jars were placed in plastic bags and incubated in the laboratory at 23 ± 3°C, 60–70% RH, and a photoperiod of 12:12 (L:D) h for 9–10 d.

Cotesia flavipes adults used in the experiments were obtained from a laboratory colony maintained on sugarcane borer larvae. This colony was established from parasitized sugarcane borer larvae collected in the Lower Rio Grande Valley and reared following the procedure of Overholt et al. (1994) for two generations before beginning the experiments. Newly formed parasitoid cocoons were collected from diet cups and placed inside cylindrical perspex containers (10 by 10 by 15 cm). Emerging adult parasitoids were allowed to feed ad libitum on 20% (vol:vol) honey:water solution offered via soaked cotton wool. A 1-cm-diameter perforation, covered with a cork, at the side of the perspex container permitted manipulation of the adult parasitoids.

Experimental Procedures. *Host Acceptance and Suitability, and Parasitoid Brood Size, Development, and Sex Ratio.* Neonate *D. saccharalis* were individually placed in covered cups containing either transgenic or nontransgenic diet. Cups were placed in trays, which were placed in incubators at 30 ± 1°C, 70 ± 2% RH, and a photoperiod of 12:12 (L:D) h. After 18 d of feeding on either diet, larvae were exposed to 24-h-old mated females of *C. flavipes* for parasitism following the hand-stinging method of Smith et al. (1993). Individual larvae were held carefully with forceps and introduced in the parasitoid container through the hole located on one side. Fifty larvae were exposed to parasitism for both the transgenic and nontransgenic diet treatments. Individual larvae were exposed to *C. flavipes* females until parasitized, which usually required 1–2 s. Only one oviposition of *C. flavipes* was allowed per larva. After being stung, individual larvae

were returned to the diet cups from which they had been taken. The diet cups were covered, placed in trays, and incubated under the environmental conditions indicated above. The diet cups were checked daily for cocoon formation starting 10 d after exposure of larvae to parasitoids. Upon formation, cocoons were placed in cylindrical containers and incubated in the same incubators as sugarcane borer larvae until emergence of adult parasitoids. These adult parasitoids were considered first generation (F_1) and were used as parents to produce a second generation (F_2) following the experimental procedure described for the F_1 generation, except that 35 sugarcane borer larvae from each diet treatment were exposed to females of *C. flavipes.* In both generations, nonpupating larvae that did not produce emerging wasps were dissected to determine whether they had been parasitized.

Host acceptance and suitability were assessed for both generations. Host acceptance was measured as the proportion of larvae effectively parasitized (=larvae not reaching the pupal stage/larvae exposed to parasitism) and host suitability as the proportion of parasitized larvae yielding parasitoid cocoons (=larvae producing parasitoid cocoons/larvae exposed to parasitism). Other data collected included host size before parasitism (=weight to the nearest 0.01 mg), brood size (=number of parasitoid cocoons and adults emerging per host), length of developmental periods (egg to cocoon and cocoon to adult), and offspring sex ratio (=male offspring/total offspring).

Rates of host acceptance and suitability were compared between the transgenic and nontransgenic diet treatments via chi-square tests of conformity within each generation (Zar 1999). The length of developmental periods, numbers of cocoons, and parasitoid adults obtained per host and egg loads of females were subjected to a two-way analysis of variance (ANOVA) with diet treatment and generation as factors. Means were separated using the Student Newman Keuls (Student–Newman–Keuls) test when significant *F* values were obtained ($P < 0.05$) (Zar 1999). Because the effect of generation was not significant for any of these parameters, data corresponding to the F_1 and F_2 generations were pooled for linear regression analysis to examine the effects of host size on numbers of cocoons and parasitoid adults emerging per host. Parallel line analysis performed by the PROC MIXED of SAS (Littell et al. 1997) was used to compare regression lines among diets. Where slopes were not significantly different, regression intercepts were compared using the LSMEANS procedure (SAS Institute 1996). Parasitoid sex ratios corresponding to each diet treatment were compared against a 1:1 ratio using a chi-square goodness-of-fit (Zar 1999). Log-likelihood ratio tests were used to compare sex ratios between diet treatments within each generation (Zar 1999). For all sex ratio comparisons, broods consisting solely of males (indicating that the parental female had not mated) were excluded from the analyses.

Egg Load and Adult Size of Female Parasitoids. Upon emergence from cocoons, 50 parasitoid females were selected randomly from each of the two generations (F_1 and F_2) and diet treatments. These females were placed in gelatin capsules and then killed by deep-freezing. Frozen females were dissected within 3 d in physiological saline (7.5 g of NaCl in 1 liter of distilled water) under a microscope. Each female was placed with its dorsal side up in a droplet of saline on a microscope slide. Two pins were used to hold each female, make lateral incisions in the distal portion of the abdomen, and extract the ovaries, which were teased apart, and the number of ovarian eggs counted. In addition, the length of the left hind tibia was measured to the nearest 0.1 μm as an index of adult size.

A two-way ANOVA with diet treatment and generation as factors was used to compare egg loads. Where the diet treatment effect was significant, means were separated using Student–Newman–Keuls test (Zar 1999). Because the effect of generation was not significant, data corresponding to the F_1 and F_2 generations were pooled for linear regression analysis to examine the effects of adult size (left hind tibia length) on egg load. Parallel line analysis performed by the PROC MIXED of SAS (Littell et al. 1997) was used to compare regression lines between diets. Where slopes were not significantly different, regression intercepts were compared using the LSMEANS procedure (SAS Institute 1996).

Longevity and Adult Size of Female Parasitoids. Upon emergence from cocoons, 60–70 parasitoid females were randomly selected from each generation and diet treatment. Adult longevity of these females was assessed at 25 ± 1°C, 65 ± 5% RH, and a photoperiod of 14:10 (L:D) h. Newly emerged females were placed individually in 7.5 by 2.5-cm glass vials plugged with cotton and provided with cotton wool soaked with 20% honey solution (vol:vol). Parasitoid mortality was recorded at 2-h intervals until all parasitoids died. In addition, the length of the left hind tibia was measured to the nearest 0.1 μm as an index of adult parasitoid size. Any individuals found dead in the honey solution or tangled in the cotton plug were excluded from analyses.

Adult longevity was analyzed via two-way ANOVA, and means were separated between treatments using Student–Newman–Keuls test (Zar 1999). In addition, data corresponding to the first and second generation were pooled and linear regression analysis was used to examine the effects of female size on adult longevity. Parallel line analysis using PROC MIXED of SAS (Littell et al. 1997) was used to compare regression lines among diets. Where slopes were not significantly different, regression intercepts were compared using the LSMEANS procedure (SAS Institute 1996).

Results

Host Acceptance and Suitability. In both generations, the proportion of sugarcane borer larvae successfully parasitized and producing parasitoid progeny was similar for both diet treatments ($\chi^2 = 0.11, P = 0.74$). Therefore, data were pooled over generations within each treatment. Sugarcane borer larvae reared on transgenic and nontransgenic diets were accepted

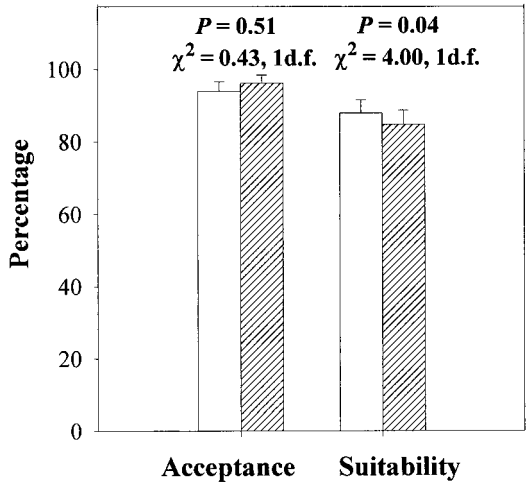

Fig. 1. Rates of host acceptance (±SD) and suitability (±SD) of *D. saccharalis* for *C. flavipes*; *D. saccharalis* were fed diet based on either transgenic (filled columns) or nontransgenic (hollow columns) sugarcane tissue. Data were pooled for two consecutive generations of *C. flavipes*.

at similar rates for oviposition by females of *C. flavipes* ($\chi^2 = 0.31$, $P = 0.57$) (Fig. 1). However, suitability of sugarcane borer larvae for parasitoid development varied with diet treatment. Significantly fewer parasitized larvae yielded parasitoid adults in the transgenic diet compared with the nontransgenic diet (Fig. 1).

Developmental Period. The total developmental period of *C. flavipes*, i.e., from egg to adult, was ≈15 d and did not vary with diet treatment ($P = 0.29$) nor generation ($P = 0.58$) (Fig. 2). However, differences were evident between partial developmental periods. Egg to cocoon developmental time was longer in the transgenic diet relative to the nontransgenic diet in the first generation but not in the second (Fig. 2). In contrast, the cocoon to adult developmental period was shorter in the transgenic diet relative to the nontransgenic diet in the first generation but not in the second (Fig. 2). Parasitoid generation had no significant effects on partial developmental periods ($P > 0.05$).

Brood Size and Host Size. Brood sizes based both on numbers of parasitoid cocoons and adults emerging per host were significantly lower in the transgenic diet relative to the nontransgenic diet in the first generation but not in the second generation (Table 1). In addition, the percentage of adults emerging from cocoons was significantly lower in the transgenic diet compared with the nontransgenic diet in both generations (Table 1). Generation effects were not significant for these variables ($P > 0.05$). Host size was greater in the transgenic diet relative to the nontransgenic diet in the first generation but not in the second generation (Table 1).

Brood sizes based on parasitoid cocoons and adults emerging per host were significantly influenced by

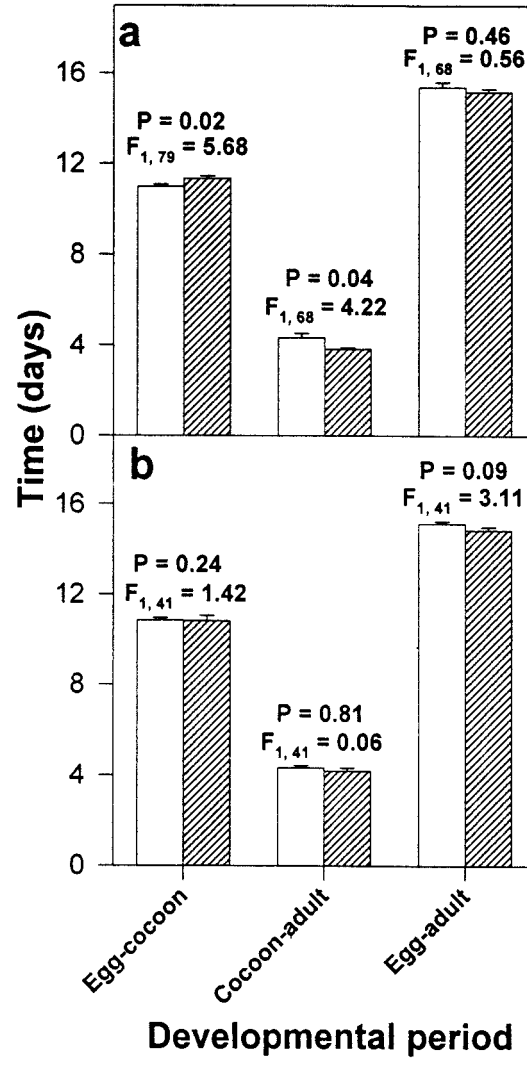

Fig. 2. Length of developmental periods (±SE) of *C. flavipes* developing on *D. saccharalis* during two consecutive generations: (a) first generation, and (b) second generation. *D. saccharalis* were fed diet based on either transgenic (filled columns) or nontransgenic (hollow columns) sugarcane tissue.

host size. Larger hosts yielded greater numbers of cocoons (Fig. 3a) and parasitoid adults (Fig. 3b) in both types of diet ($P < 0.0001$). Regression slopes corresponding to transgenic and nontransgenic diets did not differ significantly, indicating the absence of significant host mediated effects in *C. flavipes* development (Fig. 3).

Parasitoid Sex Ratios. The sex ratio of *C. flavipes* emerging from both diet treatments was female biased in both generations ($P < 0.001$) (Fig. 4). For both diet treatments, an increase in the proportion of males was evident in the second generation relative to the first generation ($P = 0.0006$). In addition, higher percentages of males occurred in the transgenic diet relative

Table 1. Host size (mean weight in mg ± SE), brood size (cocoons per host ± SE; adults per host ± SE), and adult emergence from cocoons (± SE) of *C. flavipes* when developing on *D. saccharalis* fed artificial diet based on either transgenic (T) or non-transgenic (NT) sugarcane tissue during two consecutive generations (F_1, F_2)

Generation	Treatment	Host size (mg)	Brood size (cocoons)	Brood size (adults)	% adult emergence
F_1	NT	77.9 ± 4.1a	48.1 ± 2.1b	46.9 ± 2.0b	92.7 ± 1.2b
	T	94.4 ± 4.1b	40.4 ± 2.5a	37.2 ± 2.7a	83.3 ± 3.3a
F_2	NT	71.3 ± 4.4a	46.6 ± 2.6a	43.2 ± 3.1a	92.0 ± 1.9b
	T	77.1 ± 6.0a	42.9 ± 3.2a	41.8 ± 2.9a	87.0 ± 2.0a
$F_{Generation}$		6.30*	0.04NS	0.03NS	0.01NS
$F_{Treatment}$		5.47*	4.56*	4.16*	9.5**
$F_{Generation \times Treatment}$		1.23NS	0.54NS	2.30NS	0.09NS

Means followed by the same letter in the same column for each generation are not significantly different ($P > 0.05$). NS, nonsignificant; *, significant ($P < 0.05$); **, highly significant ($P < 0.01$).

to the nontransgenic diet in the second generation but not in the first generation (Fig. 4).

Egg Load and Adult Female Size. Neither diet treatment ($P = 0.47$) nor generation ($P = 0.74$) affected mean egg loads of females of *C. flavipes* (Table 2). Data for both generations were thus pooled for linear regression analyses. For both treatments, egg load was strongly influenced by adult size of females ($P < 0.0001$) (Fig. 5a). The rates of egg load gain per increment in adult size were comparable between transgenic and nontransgenic diets ($P = 0.70$), indicating that host-diet mediated effects were not significant (Fig. 5a).

Longevity and Adult Female Size. The mean longevity of adult females of *C. flavipes* varied between 35.9 and 52.1 h depending on generation and diet treatment (Table 2). Longevity was significantly shorter in the transgenic diet relative to the nontransgenic diet in the second generation ($P < 0.0001$) but not in the first ($P = 0.37$). In addition, longevity decreased in the second generation relative to the first in the transgenic diet ($P < 0.0001$), while it did not vary with generation in the nontransgenic diet ($P = 0.65$). The longevity of adult females was influenced by their size; larger individuals living longer than smaller ones (Fig. 5b). The rate of gain of longevity with adult size of *C flavipes* differed with diet ($P = 0.045$) (Fig. 5b).

Discussion

Prior research showed no deleterious effects of GNA-transgenic sugarcane on various life history parameters of *D. saccharalis*, including survival, weight and developmental periods of larvae and pupae, and adult fecundity and egg viability (unpublished data). The results of this study, however, showed that a number of biological parameters and fitness components of its larval parasitoid, *C. flavipes* were affected by the transgenic diet. Host suitability (Fig. 1), brood sizes (Table 1), percentage adult emergence (Table 1), proportion of females among offspring (Fig. 4), and adult female longevity (Table 2) were lower in the transgenic diet relative to the nontransgenic diet. In addition, the transgenic diet resulted in an increase in the egg to cocoon developmental period in the first generation (Fig. 2), slower rate of longevity gain with parasitoid adult size (Fig. 5), and reduction in lon-

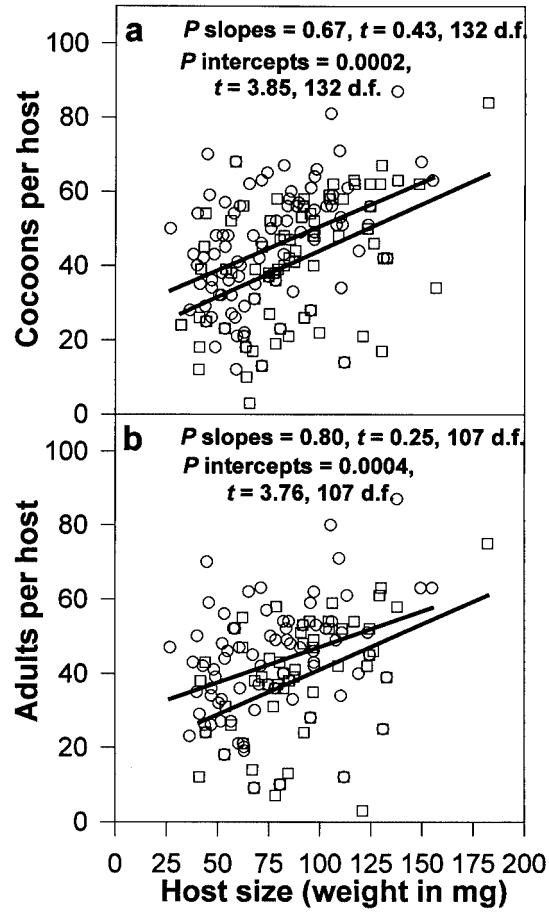

Fig. 3. Relationships between brood size of *C. flavipes* and size (weight in mg) of *D. saccharalis; D. saccharalis* were fed diet based on either transgenic (squares, upper lines) or non-transgenic (circles, lower lines) sugarcane tissue. The relationships are described by the equations: (a) non-transgenic, $y = 26.85 + 0.24x$ ($P < 0.0001$, $r^2 = 0.18$, 92 d.f.), and transgenic, $y = 19.05 + 0.25x$ ($P < 0.0001$, $r^2 = 0.21$, 73 d.f.), and; (**b**) non-transgenic, $y = 27.91 + 0.19x$ ($P = 0.001$, $r^2 = 0.13$, 80 d.f.), and transgenic, $y = 16.68 + 0.24x$ ($P = 0.0007$, $r^2 = 0.19$, 58 d.f.).

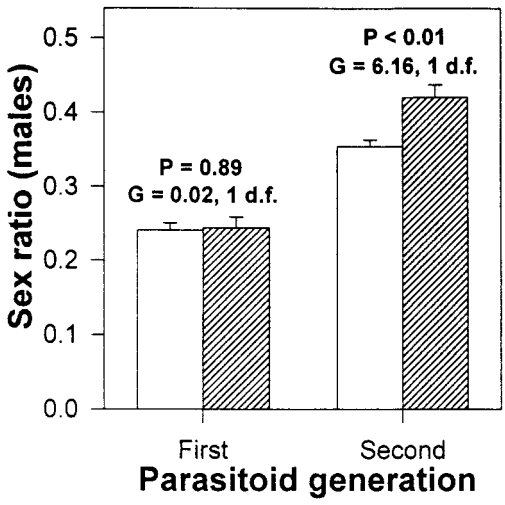

Fig. 4. Offspring sex ratio of *C. flavipes* emerging from *D. saccharalis* during two consecutive generations; *D. saccharalis* were fed diet based on either transgenic (filled columns) or nontransgenic (hollow columns) sugarcane tissue.

gevity between generations (Table 2). In contrast, the transgenic diet did not affect host acceptance rate, egg to adult developmental period, and egg load of *C. flavipes.* The significant effects of the transgenic diet on *C. flavipes* found in this study are important because the GNA levels in the transgenic diet (≈0.49%) were equivalent to ≈50% the level present in transgenic sugarcane plants (unpublished data).

Similar to this study, Bell et al. (1999) did not find a significant difference in host acceptance rates between parasitoids offered hosts feeding on diet either containing or lacking GNA. *Eulophus pennicornis,* a gregarious ectoparasitoid, oviposited on ≈65% of *L. oleracea* fed diet either containing or lacking GNA (Bell et al. 1999). This result is not surprising in the case of *C. flavipes,* because (1) there is no a priori reason for ovipositing females to detect anti-feedant compounds present in a host, (2) preovipositional probing by ovipositing females is limited because oviposition requires 1–2 s and risk of host attack-related mortality is high (Potting et al. 1999), and (e) prior studies demonstrated that ovipositing females show little discrimination among suitable hosts fed different plant cultivars and between suitable and unsuitable hosts (Okech and Overholt 1996, Boica et al. 1997, Potting et al. 1997). Egg to adult developmental periods were similar between diet treatments in this study, which is consistent with results reported by Bell et al. (1999). However, this result is inconsistent with those of other studies in which parasitoids developing on hosts reared on resistant or nonpreferred plants have developmental periods longer than those on hosts reared on susceptible or preferred plants (Karowe and Schoonhoven 1992, Riggin et al. 1992, Souissi and le Ru 1998). Similar to the current study, Fuentes-Contreras et al. (1996) found that the egg to pupa developmental period of the parasitoid *Aphidius rhopalosiphi* DeStefani-Perez increased in resistant wheat cultivars expressing high levels of hydroxamic acids. This suggests that host plant resistance, via GNA or hydroxamic acids, affected parasitoid development during the active feeding stage, i.e., the larval stage; whereas parasitoid pupae developed at normal rates. Egg load was not affected in *C. flavipes* in the transgenic diet treatment, which is consistent with the results of Bell et al. (1999). Moreover, mean egg loads of *C. flavipes* in both diet treatments in this study are similar to those reported previously in other studies (Wiedenmann et al. 1992, Potting et al. 1997). The small to nil differences in developmental times and egg loads between *C. flavipes* reared on hosts fed transgenic or nontransgenic diet were likely a result of the low levels of GNA present in the transgenic diet.

Previous studies showed that sugarcane borer developed faster and grew larger on diet containing GNA versus diet free of GNA (unpublished data). This suggested that GNA, at the level tested, contributed to sugarcane borer growth and development. Other studies involving proteinase inhibitors showed similar results, suggesting that below specific thresholds, these compounds may complement the protein content of the diet or host plant thus favoring herbivore growth and development (de Leo et al. 1998). Prior studies showed that GNA ingested by larvae of *L. oleracea* accumulates in the gut and is present in the hemolymph and thus is readily available to parasitoids and predators feeding on these larvae (Fitches et al. 1997, Fitches and Gatehouse 1998). Therefore, the lack of effects of the transgenic diet on egg loads and developmental times coupled with the significant effects on host suitability and adult emergence rates, brood sizes,

Table 2. Egg load (±SE) and longevity (hours) (±SE) of *C. flavipes* developing on *D. saccharalis* fed artificial diet based on either transgenic (T) or non-transgenic (NT) sugarcane tissue during two consecutive generations (F_1, F_2)

Generation	Treatment	Egg load	*n*	Longevity (h)	*n*
F_1	NT	124.0 ± 4.8a	45	49.7 ± 2.8a	40
	T	119.6 ± 4.0a	45	52.1 ± 2.3a	41
F_2	NT	124.7 ± 5.5a	45	48.2 ± 1.7b	40
	T	122.1 ± 5.1a	40	35.9 ± 1.7a	40
$F_{Generation}$		0.11NS		16.54**	
$F_{Treatment}$		0.52NS		5.10*	
$F_{Generation \times Treatment}$		0.56NS		11.42**	

Means followed by the same letter in the same column for each generation are not significantly different ($P > 0.05$). NS, non significant; *, significant ($P < 0.05$); **, highly significant ($P < 0.01$).

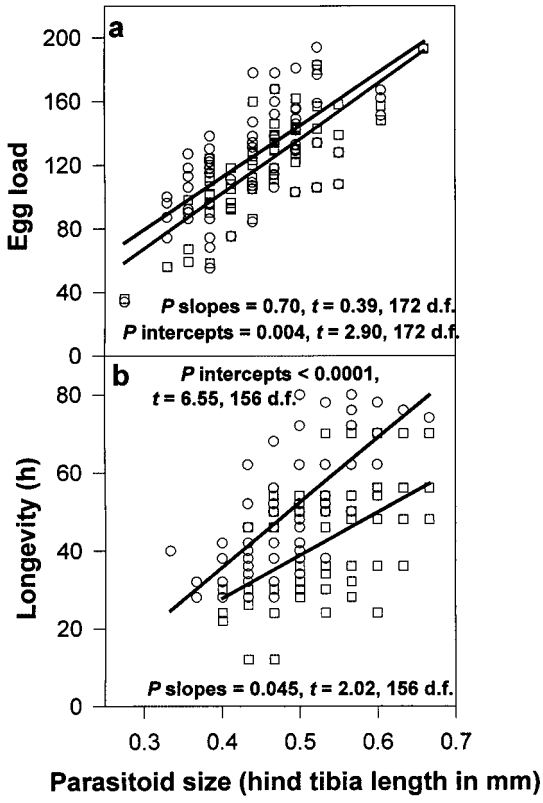

Fig. 5. Relationships between adult size (length of hind tibia in mm) and (**a**) egg load and (**b**) longevity (in hours) in *C. flavipes* developing on *D. saccharalis; D. saccharalis* were fed diet based on either transgenic (squares, upper lines) or non-transgenic (circles, lower lines) sugarcane tissue. The relationships are described by the equations: (**a**) non-transgenic, $y = -19.3 + 328.1x$ ($P < 0.0001$, $r^2 = 0.52$, 89 d.f.), and transgenic, $y = -36.6 + 345.8x$ ($P < 0.0001$, $r^2 = 0.62$, 84 d.f.), and; (**b**) non-transgenic, $y = -30.85 + 166.24x$ ($P < 0.0001$, $r^2 = 0.48$, 79 d.f.), and transgenic $y = -8.83 + 95.3x$ ($P < 0.0001$, $r^2 = 0.18$, 79 d.f.).

sex ratios, and longevity of *C. flavipes* suggests that the level of GNA present in the diet may have been below the threshold necessary to affect the former parameters while it was sufficiently high to affect the latter ones in this parasitoid.

Several possible mechanisms may explain the significant effects observed in *C. flavipes* fed transgenic diet, including potential differences in physiological age between sugarcane borer larvae in both treatments. A prior study showed that sugarcane borer larvae developed faster and pupated 2–3 d earlier when fed diet containing GNA versus diet free of GNA (unpublished data). Thus, larvae exposed to *C. flavipes* females for oviposition in the transgenic diet treatment may have been physiologically older, i.e., closer to pupation, than those in the nontransgenic diet treatment. Tanwar and Varma (1996) reported that third-to-fifth-instar larvae of *Chilo auricilius* Dudgeon were more suitable than older larvae for development of *C. flavipes*. Older larvae may represent lower-quality hosts for developing *C. flavipes* or may mount stronger immune responses, thus leading to higher parasitoid larval mortality. Differences in quality between younger and older hosts may also explain the greater proportion of male progeny in the transgenic diet treatment. A higher proportion of female eggs may have been laid in sugarcane borer larvae fed nontransgenic diet as a result of their younger physiological age. However, this explanation is unlikely because significant host instar-related differences in the sex ratio do not occur in the congener *Cotesia glomerata* (L.) (Harvey 2000), and females of this species are incapable of adjusting offspring sex ratios at the time of oviposition (Tagawa 2000). Moreover, a significant correlation between brood size and sex ratio is absent in *C. glomerata* (Ikawa and Okabe 1985). Thus, the significantly greater proportion of males in the transgenic treatment during the F_2 generation may be a result of greater developmental mortality of females of *C. flavipes* in this treatment relative to the nontransgenic diet treatment. However, confirmation of such differences in developmental mortality requires detailed experiments specifically designed to address this question.

The results presented here and reported by Bell et al. (1999) and Birch et al. (1999) show that GNA effects on natural enemies are variable and likely dependent on GNA levels in host plants and host or prey individuals. Bell et al. (1999) detected few and inconsistent negative effects of GNA in the parasitoid *E. pennicornis*, whereas brood size was higher on hosts fed diet containing GNA relative to a control diet. In contrast, Birch et al. (1999) reported significant negative effects of GNA, persisting for up to 3 wk after treatment, on various fitness parameters (fertility, egg viability, and longevity) of the predator *A. bipunctata.* In the current study, with a low level of GNA present in the host's diet, a number of fitness parameters were negatively though marginally affected in *C. flavipes*, whereas others were unaffected. The conflicting results obtained in these three studies involving GNA may be explained in part by the differing relationships between the natural enemies and herbivores involved in each study, and the different amounts of GNA consumed by the natural enemies. A predator species was involved in the study by Birch et al. (1999), while idiobiont and koinobiont (hosts continue growing after parasitism) parasitoid species were involved in the study by Bell et al. (1999) and the current study, respectively. Predatory ladybeetles in the first study were fed prey items for 12 d and likely accumulated high levels of GNA. In the current study, it is likely that developing *C. flavipes* also accumulated high levels of GNA, because parasitized hosts continued feeding for ≈10–12 d before succumbing to parasitism. In contrast, developing *E. pennicornis* likely accumulated little GNA because feeding is greatly reduced in parasitized hosts (Marris and Edwards 1995, Weaver et al. 1997).

Potential side effects of transgenic insecticidal crop cultivars on natural enemies and other nontarget or-

ganisms are increasingly being examined (Hilbeck et al. 1998, Bell et al. 1999, Birch et al. 1999, Losey et al. 1999, Schuler et al. 1999, Jesse and Obrycki 2000, Wraight et al. 2000, Couty et al. 2001). Such studies are necessary if these cultivars are to become widespread in integrated pest management tactics. A number of researchers have hypothesized likely negative interactions between transgenic cultivars and parasitoids of target pests. For instance, it is suggested that transgene proteins may be directly or indirectly toxic to parasitoids and/or the host location process of parasitoids may be altered in transgenic crops or for hosts feeding on these crops (Schuler et al. 1999). The significant negative effects of GNA on several fitness parameters of *C. flavipes* observed in this study are important, because GNA expressed at the same level (≈0.49% in the diet) had nil to positive effects on sugarcane borer (unpublished data). Thus, it is imperative to consider potential impacts on biological control of sugarcane borer by *C. flavipes* before releasing GNA-expressing cultivars for commercial use in south Texas and other areas where this parasitoid is known to suppress this pest. Although conclusions cannot be drawn at this stage concerning how the present findings might translate in the field, it is possible that significant effects on sugarcane borer biological control may occur because the level of GNA expressed in transgenic sugarcane plants (0.9% of toal protein) is twice that expressed in the diet used in the current study (0.49% of total protein) (unpublished data). Field studies are underway to examine more closely the impact of GNA-expressing transgenic sugarcane on the population dynamics of *C. flavipes* and sugarcane borer.

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